Technical Data Sheet

FITC Annexin V Apoptosis Detection Kit II

Product Information

Material Number:	**556570**
Component:	**51-65871A**
Description:	Purified Recombinant Annexin V
Size:	100 µg (1 ea)
Concentration:	0.5 mg/ml
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.
Component:	**51-65874X**
Description:	FITC Annexin V
Size:	0.5 ml (1 ea)
Vol. per Test:	5 µl
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-66211E**
Description:	Propidium Iodide Staining Solution
Size:	2.0 ml (1 ea)
Vol. per Test:	5 µl
Storage Buffer:	Aqueous buffered solution containing no preservative.
Component:	**51-66121E**
Description:	10X Annexin V Binding Buffer
Size:	50 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing no preservative.

Description

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenence of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including FITC. This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis. Since externalization of PS occurs in the earlier stages of apoptosis, FITC Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

FITC Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with FITC Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (PI negative, FITC Annexin V positive). Viable cells with intact membranes exclude PI, wheras the membranes of dead and damaged cells are permeable to PI. For example, cells that are considered viable are FITC Annexin V and PI negative; cells that are in early apoptosis are FITC Annexin V positive and PI negative; and cells that are in late apoptosis or already dead are are both FITC Annexin V and PI positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both Annexin-FITC and PI. However, when apoptosis is measured over time, cells can be often tracked from FITC Annexin V and PI negative (viable, or no measurable apoptosis), to FITC Annexin V positive and PI negative (early apoptosis, membrane integrity is present) and finally to FITC Annexin V and PI positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both FITC Annexin V and PI positive, in of itself, reveals less information about the process by which the cells underwent their demise.

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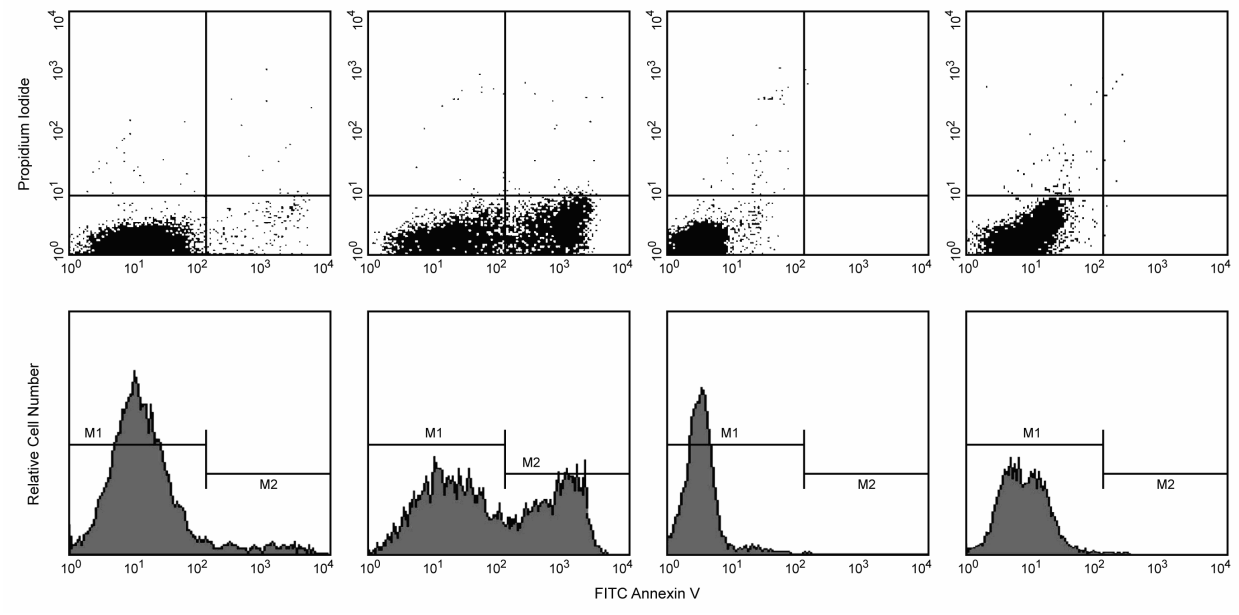

Flow Cytometric Analysis of FITC Annexin V staining and blocking. *Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were either untreated (top/bottom far left and middle-right panels) or were induced to undergo apoptosis by treatment with camptothecin (12 µM, 4 hours) (top/bottom middle-left and far right panels). Cells were then incubated with FITC Annexin V in a buffer containing propidium iodide (top/bottom far left and middle left panels) or with purified recombinant Annexin V (top/bottom far right and middle-right panels) in order to block FITC Annexin V binding sites prior to adding FITC Annexin-V. After camptothecin treatment (4 hours), a population of cells was FITC Annexin V positive (bottom middle-left panel, M2). FITC Annexin V staining was blocked when cells were first incubated with purified recombinant Annexin V (bottom far right panel, M2). As expected, the cell population that was not treated was primarily Annexin V negative (bottom far left panel, M1). The small number of Annexin V positive cells in the untreated population likely represents a basal level of apoptosis (bottom far left panel, M2).*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Store all components except the recombinant Annexin V (component no. 51-65871A) at 4°C and protected from prolonged exposure to light. Do not freeze.

Store the recombinant Annexin V (component no. 51-65871A) at -80°C avoiding repeated freeze/thaw cycles to minimize the risk of protein degradation.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

FITC Annexin V is a sensitive probe for identifying apoptotic cells, binding to negatively charged phospholipid surfaces (Kd of ~5 x 10^-2) with a higher affinity for phosphatidylserine (PS) than most other phospholipids. FITC Annexin V binding is calcium dependent and defined calcium and salt concentrations are required for optimal staining as described in the FITC Annexin V Staining Protocol. **Investigators should note that FITC Annexin V flow cytometric analysis on adherent cell types (e.g HeLa, NIH 3T3, etc.) is not routinely tested as specific membrane damage may occur during cell detachment or harvesting. Methods for utilizing Annexin V for flow cytometry on adherent cell types, however, have been previously reported (Casiola-Rosen et al. and van Engelend et al.).**

INDUCTION OF APOPTOSIS BY CAMPTOTHECIN

The following protocol is provided as an illustration on how FITC Annexin V may be used on a cell line (Jurkat).

Materials

1. Prepare Camptothecin stock solution (Sigma-Aldrich Cat. No. C-9911): 1 mM in DMSO.
2. Jurkat T cells (ATCC TIB-152).

Procedure

1. Add Camptothecin (final conc. 4-6 µM) to 1 x 10^6 Jurkat cells.
2. Incubate the cells for 4-6 hr at 37°C.
3. Proceed with the FITC Annexin V Staining Protocol to measure apoptosis.

FITC ANNEXIN V STAINING & BLOCKING PROTOCOL

FITC Annexin V is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. It relies on the property of cells to lose membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment. Annexin V is a calcium-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. Cells that stain positive for FITC Annexin V and negative for PI are undergoing apoptosis. Cells that stain positive for both FITC Annexin V and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both FITC Annexin V and PI are alive and not undergoing measurable apoptosis.

Reagents

1. FITC Annexin V (component no. 51-65874X): Use 5 µl per test.
2. Propidium Iodide (PI) (component no. 51-66211E) is a convenient, ready-to-use nucleic acid dye. Use 5 µl per test.
3. 10X Annexin V Binding Buffer (component no. 51-66121E): 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM CaCl2. For a 1X working solution, dilute 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water.
4. Purified Recombinant Annexin V (component no. 51-65871A): Use 5-15 µg per test. Researchers are encouraged to titrate the reagent for optimal results.

Staining

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^6 cells/ml.
2. Transfer 100 µl of the solution (1 x 10^5 cells) to a 5 ml culture tube.
3. Add 5 µl of FITC Annexin V and 5 µl PI.
4. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
5. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

Blocking

As an optional control, researchers may pre-incubate cellular samples with purified recombinant Annexin V (component no. 51-65871A). By blocking FITC Annexin V binding sites, FITC Annexin V specific binding can be demonstrated as illustrated in the figure.

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^6 cells/ml.
2. Transfer 100 µl of the solution (1 x 10^5 cells) to a 5 ml culture tube.
3. Add 5-15 µg of purified recombinant Annexin V. The amount of purified recombinant Annexin V required to saturate binding sites may vary according to cell type and stage of apoptosis. In some cases, investigators may need to reduce the number of cells to 0.5 x 10^5 and still add 5-15 µg of recombinant Annexin V to obtain optimal results. Titration is strongly recommended.
4. Gently vortex the cells and incubate for 15 min at room temperature.
5. Add 5 µl FITC Annexin V and 5 µl PI.
6. Gently vortex the cells and incubate for 15 min at room temperature in the dark.
7. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry as soon as possible (within 1 hr).

SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY

The following controls are used to set up compensation and quadrants:

1. Unstained cells.
2. Cells stained with FITC Annexin V (no PI).
3. Cells stained with PI (no FITC Annexin V).

Other Staining Controls:

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with FITC Annexin V and/or FITC Annexin V and PI. It is important to note that the basal level of apoptosis and necrosis varies considerably within a population. Thus, even in the absence of induced apoptosis, most cell populations will contain a minor percentage of cells that are positive for apoptosis (FITC Annexin V positive, PI negative or FITC Annexin V positive, PI positive).

The untreated population is used to define the basal level of apoptotic and dead cells. The percentage of cells that have been induced to undergo apoptosis is then determined by subtracting the percentage of apoptotic cells in the untreated population from percentage of apoptotic cells in the treated population. Since cell death is the eventual outcome of cells undergoing apoptosis, cells in the late stages of apoptosis will have a damaged membrane and stain positive for PI as well as for FITC Annexin V. Thus the assay does not distinguish between cells that have already undergone an apoptotic cell death and those that have died as a result of necrotic pathway, because in either case the dead cells will stain with both FITC Annexin V and PI.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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